STUDIES ON THE EXPERIMENTAL INCIDENCE OF PERICOSTAL TUBERCULOSIS

by

MASAKATSU YAMAMOTO

From the Second Surgical Clinic of Kyoto University Hospital
(Director : Prof. Dr. YASUMASA AOYAGI)
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Concerning the mode of development of pericostal tuberculosis numerous clinical studies have been made by SOULIGOUX, OMURA, INOKO, SAITO, KAUFMAN, TAKEUCHI and SHIMIZU etc. Search of the literature has revealed no unequivocal record of the experimental incidence of pericostal tuberculosis except that of BURKE. He, like KAUFMAN, postulated the lymphnode origin of pericostal tuberculosis and was able to induce the tuberculous lesions in the sternal lymphnodes of guinea pigs. No study and description of the histopathologic changes in these affected lymphnodes and the lymphatics of chest wall was published.

However, in our clinic, since it was designated as pericostal tuberculosis by Prof. INOKO in 1908, many studies have been made from the clinical point of view and TAKEUCHI has postulated two modes of pathogenesis as follows: (a) by way of the newly developed lymphatics in pleuritic adhesions, tubercle bacilli from the lung lesions invade the lymphatic system of the chest wall ; (b) tubercle bacilli in the pleuritic exudates are absorbed from the parietal pleura into the lymphatic system of the chest wall and then may settle there to cause disease.

The present study was undertaken in order to investigate the concept of the latter view in laboratory animals.

METHOD AND MATERIAL

1). Bacterial cultures and dosage. The following strains of tubercle bacilli were used: the virulent human strain H37Rv, the FRANKFURT strain, and unclassified strains of high virulence which had been isolated from human sputum. Each animal was inoculated with a suspension of tubercle bacilli in isotonic saline. Varying amounts, ranging from 1-5 mg of a month old culture grown in KIRSCHNER's culture medium was usually used. Occasionally, 7-day-old cultures grown in DUBOS's culture medium were used. In order to remove the larger clumps of bacilli, the cultures were centrifuged at a slow speed for about 15 minutes. The optical density of each supernant was measured with the TORIKATA'S praecipitometer. By diluting with the uncultured DUBOS's culture medium, all supernates were adjusted to an optical density of 10 degree. This density correspond with about 5mg of tubercle bacilli.

2). Animals. Albino rabbits weighing approximately 2kg were used.

3). Technical procedures. The 7 series of experiments indicated in Table 1 were performed. The 7 groups of rabbits used were designated A, B, C, D, E, F,

Table I

SUMMARY OF STUDIES ON EXPERIMENTAL INCIDENCE OF PERICOSTAL TUBERCULOSIS IN EACH SERIES

Group	Sensibilization		Interval	Reinoculation		Incidence of tuberculous lesions			
	Route	Dose		Route	Dose	Lungs	Sub-pleura	Lymphnodes sternal	Lymphnodes intercost.
A.	subcutan.	5mg/kg	3w--5w.	intrapleural.	5mg/kg	+	++	+	±
B.	trachea.	〃	〃	〃	〃	+	+	+	±
C.	intrapleural.	〃	〃	trachea	〃	++	+	+	±
D.	〃	〃	〃	intraabdominal.	〃	±	±	++	±
E.	intraabdominal.	〃	〃	intrapleural.	〃	±	+	++	±
F.	〃	〃	〃	intraabdominal.	〃	−	−	++	±
G.	intrapleural.	〃	〃	intrapleural.	〃	±	+	+	±

and G. In order to inject accurately in the pleural space (at the VIII intercostal space on the right armpit-line), a three-way petcock that was connected to a water manometer, a thin blunt pneumothorax-needle and a syringe were employed.

Animals were bled to death at various intervals. Some at the end of the first and second weeks and at 1,2 and 3 months after inoculation. Prior to this healthy animals were sacrificed to investigate histologically the lymphatic drainage system of the thorax in a normal condition.

Routine gross and microscopic studies were performed. The lung, parietal pleura, mediastinum, peritoneal diaphragm and lymphnodes in the thorax—especially Lgll.intercostales dorsales, Lgll. sternales craniales, mediae et caudales, Lgll. oesophageae, Lgll. interpericard diaphragmaticae, Lgll. paratracheales — were examined in all animals. The liver, spleen, Lgll. aortae thoracales caudales lat. et dorsales, Lgll. mediastinales dorsales. A. subclav. sinistrae, Lgll. mediastinales ventrales. V. cavae. craniales dextrae, Lgll. arcis aortae, Lgll. tracheobroncheales dextrae ant. and post. were examined only occasionally. After fixation, the tumor and chest wall were carefully removed enbloc with the parietal pleura. The specimen was decalcified with 4% HCl. Fixation was accomplished by 10% neutral formalin, although CARNOY's solution and absolute alcohol and Methanol are sometimes used. These materials were embedded in paraffin or celloidin. Sections were cut at 6 to 15 μ and occasionally serially.

For the purpose of morphologic study of the pleura parietalis, mediastinum and diaphragm, care was taken to peel them out from the underlying tissue thinly as possible and they were streched over a clean glass slide.

The section were usually stained with hematoxylin and eosin. Special stains included WEIGERT's elastic fiber stains, BIELSCHOWSKY-MARESCH silver method, anilin fuchsin stain for tubercle bacilli, picric acid-fuchsin (VAN GIESON) methylgreen pyronin stain, MAY-GIEMSA stain, peroxydase reaction, fibrin stain and ASAN'S stain.

ANATOMICAL REVIEW

Numerous experimental studies have been made on the extent and routes of drainage of the lymphatic system of the pleural and abdominal cavity. But, viewed from a systematical angle, none but the studies here to be reviewed deserve the name. The following information has been brought to light by KIHARA and his scholars.

1) MACULA CRIBRIFORMIS

G. TESIMA (1932) found that India ink (a suspension of finely divided carbon) injected into the peritoneal cavity was absorbed chiefly from the diaphragmatic peritoneum. About 10 minutes after injection they revealed carbon in the lymphatic channels of the diaphragm, in the internal mammary lymphatics and thoracic duct. The greatest amount was found in these sites about 30 minutes after injection and 6 hours later no India ink was found in these lymphatic vessels. Furthermore, he pointed out that about 50 minutes after injection, the injected India ink readily escaped from the internal mammary lymphatics to the surrounding soft tissues (such as fatty tissues) and then carbon particles were phagocytosed by polymorphnuclear leucocytes or histiocytes. At the same time he observed that these carbon particles were absorbed through the intercellular cement substances of the diaphragmatic peritoneal endothelium.

This study concerned with the absorption from the peritoneal cavity, in briefly speaking, suggest the absorption time and definite absorption site and demonstrates the extravascular leakage phenomenon of the internal mammary lymphatics.

Afterwards, OGO (1934) reported that the absorption from the pleural cavity of rabbit was largely from the parietal and mediastinal pleura and especially the anterior portion of the parietal pleura. Lymphatic channels here were visualized with India ink to the tributary lymphnodes. On the other hand, the mediastinal pleura was stained black but underlying lymphatic vessels were not visualized. Absorption from the visceral and diaphragmatic pleura were never seen. OGO explained as follows: the local variability of absorptive ability was attributed to functional difference between pleural endothelium and lymphatic endothelium.

TEI (1937) found morphologically the so-called stomata situated only in the absorptions site between diaphragmatic endothelium and lymphatic capillaries.

After that the problem of the extravascular fluid path in the tissue spaces (in several parts of the laboratory animals) were systematically studied by many investigators under Prof. KIHARA and finally on the study of so-called Kraterförmige Stigmata of the pleural and peritoneal membrane of amphibia it was found that the constant existence of the reticulum fiber as a constitutional element in the absorptions passage from the serous cavity to the lymphatic vessels.

Following this study of the absorptions sites from the serous cavity to the lymphatic vessels, TSUBOUCHI (1950) discovered sieve-like constitutions that were formed by both collagen and reticulum fibers in the subendothelial connective tissues of the parietal pleura or diaphragmatic peritoneum.

As this constitution has a spotlike distribution over the parietal pleura or

diaphragmatic peritoneum in mediastinal pleura spread out continuously, the former was coined by Prof. KIHARA macula cribriformis and the latter membrana cribriformis (Fig. 11. 12. 13).

On the parietal pleura, the distribution of macula cribriformis was richest in the anterior or posterior under portion, and next to this in the anterior upper portion. Very few are located in the posterior upper and lateral portion, and only in the intercostal spaces but none on the ribs.

Besides, this distribution was consistant through human and mammals in each series but its constitution was more or less different from each other.

2) EXTRAVASCULAR LEAKAGE PHENOMENON

The character of the extravascular leakage phenomenon of retrosternal lymphatic vessels--that had been disoovered by G. TESIMA (1932) afterwards confirmed by TEI (1937) and ASADA (1947)--was still attributed to the special function of these lymphatic endothelium to excrete foreign bodies outwards from these lymphatic vessels.

Afterwards in the anatomical institute of Prof. KIHARA, the ontogenetic and phylogenetic studies concerning the lymphatic apparatuses belonging to the lymphatic and venous system were systematically and consistently investigated. Consequently, in the lymphvascular system of all vertebrate, it was noticed that primitive lymphatic apparatuses appeared in the form of subendotheliar lymph-infiltration or lymphnodule which was based upon reticulum cells and fibers. Furthermore, it was confirmed by many of KIHARA's scholars that in all animals under birds it appeared in this form, but it was still dotted, along with already-existing lymphnode, this primitive lymphatic apparatuses, mingling among them, over every places of all mammalia.

In regards to this, on the study of lymphatic vessels of wild duck KOWA(1934) found that the extravascular leakage phenomenon of India ink took place only in sites of such lymphatic apparatuses. Availing of this opportunity, KOWA(1934) reinvestigated the foregoing TESIMA's experiment and consequently found that the leakage phenomenon of India ink took place only in sites of such apparatuses and then the leaked India ink spread along reticulum fibers over the surrounding connective, adipose, or muscle tissues etc.

After that this problem was reexamined by TUBOUCHI and then these carbon particles were seen only in networks of reticulum fibers making the ground substances of these lymph apparatuses, and having no relation to lymphocytes.

Finally in concerning with this problem, it was concluded that the extravascular leakage phenomenon of India ink is possible in such subendotheliar places that abound in reticulum fibers in spite of the lack of lymphatic apparatuses but also carbon particles spread over surrounding only along the reticulum fiber.

3) MILK-SPOTS ON THE PARIETAL PLEURA

The small quantity of fluid present in the normal pleural cavity or peritoneal cavity, contains very few cells, and these consist almost entirely of monocytes. These isolated tissue monocytes tend to form the milk-spotlike clumps on the parietal

pleura and these milk-spots on the parietal pleura look like the source of free tissue monocytes in the pleural cavity as the milk-spots on the omentum are the source of these cells in the abdominal cavity.

HORII and TAMAKI stated that these tissue monocytes are absorbed into the subpleural lymphatic vessels, and go directly into the blood by passing through the endothelial walls of the postcapillary veins of the lymphnodes. The complete lack of so called matured monocytes that are confusable with histiocytes in these peripheral lymphatic vessels (peripheral afferent vessels) is characteristic.

More recently, AKAZAKI and KOJIMA have noted that in the mouse, 1 cc of the normal pleural fluid contains about 30,000 intrapleural free cells that consist of phagocytic cells as follows: tissue histiocytes (85%), lymphocytes (11%), and small number of mastcells, mesothelial cells, polymorphnuclear cells. As the source of origin of these phagocytic cells they pointed out the intrapleural milk-spotslike cell accumulations.

EXPERIMENTAL RESULT

GROSS EXAMINATION:

Necropsy examination in each series revealed pleuritis or peritonitis in varying degree. In some the parietal pleura was slightly or closely adherent to the visceral pleura. The most prominent feature indicated in Table 1 was the presence of tuberculous masses in varying size and degree within the chest wall. The masses were usually few in number and were sharply circumscribed, presented a greyish-yellow color were usually isolated, but occasionally showed confluent and conglomerate masses and tendency to caseation. In only one instance was liquefaction observed.

Inspection of Table 1, in which the results are tabulated, reveals certain differences in predilections site and structure on the incidence of these foci between each group. In the anterior portion of the chest wall, the larger number of them originated in the sternal lymphnodes, and at least in part were located in adipose tissue beneath the parietal pleura or along the internal mammary lymphatics (Fig. 1).

In the dorso-lateral portion of chest wall, they were chiefly located in the intercostal muscles or adipose tissue suggesting close relation to parietal pleura (Fig. 2).

In some instances could be revealed the tuberculous mass formation between both mediastinum (Fig. 3).

Then the histological observations to be recorded herein will, for convenience, be presented under the following category: the changes in the lymphnodes, in the adipose tissue, in the macula cribriformis, and concerning the pleural adhesions.

LYMPH NODES:

One of the most characteristic changes shown in the anterior portion of the chest wall was the change in the sternal lymph nodes, especially in the superior sternal lymph nodes.

1) Lgll. sternales craniales.

As mentioned, the most pronounced feature of the gross appearance was enlargement from two to ten times of the sternal lymphnodes which generally showed a uniform appearance. The largest measured 1.2 by 0.9 by 0.8 cm. They were round or oval and occasionally irregular or spherical and tended to form the congulomerate nodes. The nodes situated in the upper two spaces on each side lay in the fatty areolar tissue upon the endothoracic fascia, and in the plane of, or superficial to, the plane of the internal mammary artery, vein and lymphatics, beneath the internal intercostal muscle.

But no instance revealed liquefaction. Histologically, in contrast with that, each different type of change in sternal nodes between inoculated and non inoculated sites could be seen in Group D and E animals and the same type of changes in both sites (could be revealed) in Group F animals.

With few exceptions, microscopically, most of the sternal nodes were involved. The most severely affected were seen in the cases of Group D, E and F.

In the cases of Group D and E, the sternal nodes removed for autopsy 3 monthes after reinoculation, revealed as the most prominent feature, a massive caseation which involved almost all the lymph nodes (Fig. 4. 5. 6).

In one instance as shown in figure 4 there were not so much as a peripheral zone of epitheloid cells close around these large caseous foci.

In other instances as indicated in figure 5 and 6 one found a few epitheloid cells or an occasional giant cell in the periphery of the caseous foci and merely peripheral remnants of lymphoid follicles that was fallen into oppressed atrophy. But in a small lymphnodes adjacent to this large caseous lymphnodes shown in figure 6, one could find several isolated productive tubercle foci with marked central caseation, more or less irregular or spherical shaped, serrated borders and were chiefly located in the parts from the medullary sinuses to cord.

In some instances of Group D and E, 2 weeks after reinoculation, a type of diffuse caseous lymphosinuitis that widely invaded the almost whole lymphosinus system was found. Sections stained with anilinfuchsin revealed acid-fast bacilli occuring singly in clumps.

On the other hand, the superior sternal lymphnodes demonstrated only sinus cattarrh or scattered, sharply defined, small epitheloid cell tubercles which were seldom confluent with no caseation, particulary in the medulla.

When the tubercle bacilli grown in the Dubos's culture medium separated from human sputum were used, usually they showed tending to severe, although some times quite extensive, tuberculous changes as above mentioned.

Next to this the changes shown in Group F, the various stages in the development of the tuberculous foci could be seen, from the massive caseation identical with figure 4, 5, 6 or diffuse caseous lymphosinuitis, to the sinus cattarrh. But in the most instances, the normal structure of these nodes was largely obscured by a tuberculous process consisting of many, discrete, or slightly confluent epitheloid cell tubercles or isolated productive foci of fairly uniform size that chiefly laid in the

medullary sinuses partly encroaching upon the medullary cords (Fig. 7). While in others, the most striking changes were those in the sinus, there was a considerable degree of epitheloid cell proliferation with occasional giant cells, of patchy distribution and affecting predominantly the medullar sinuses and more or less the adjacent parts of the medullary cords and intermediate sinuses and lying free in the lumina of sinuses. With appropriate stains, no acid-fast bacilli could be seen in these lesions. This figure fits the concept designated as diffuse epitheloid cell proliferating lymphosinuitis in which so-called large cellular hyperplasia (ZIEGLER) may be more conspicuous (Fig. 8).

The rabbits sacrificed from Group G showed the same type of lesions, but these were much less extensive.

In generally, Group A, B and C, showed similar pathologic changes to a far lesser degree. The most commonly revealed changes in these groups was a slight or moderate dilatation of the sinuses, indicating proliferation, swelling and desquamation of the sinus endothelium, and histiocytes, large mononuclears, and lymphocytes lying free in the lumina of sinuses, and occasionally with a small quantity of fibrin deposites. This changes correspond to so-called sinus catarrh. Sometimes found were the little isolated tuberculous foci chiefly situated in the medullary sinus partly encroaching upon the medullary cords. But in these groups, no instance could be revealed of such severe and extensive change as shown in figure 4. 5. 6.

2) Lgll. sternales media or caudales.

Of particular characteristic was the involvement as a sequel of peritoneal injection (Group D. E. F). Lymphnodes were small, and no correlation with the lesions of the superior sternal lymphnodes could be made. The microscopic examinations of Group D, E and F showed the same pathology as that noted in the superior sternal nodes of these Groups and was unable to disclose the congulomerate formation. The animals in other Groups, showed somewhat less pathology as compared with the superior sternal nodes in same groups.

3) Lgll. intercostales dorsales.

Dorsal intercostal lymphnodes over 1 mm. in size were searched for and, when found, were examined histologically. In generally, the microscopic changes in these lymphnodes were not remarkable. The most commonly disclosed changes were only a few small isolated epitheloid tubercles, some of which had central caseation. Occasionally a marked sinues catarrh was found, but, it could not be compared with the severe chanegs mentioned above. In some instances, no appreciable reaction occured.

From this result, it seems to me that the dorsal intercostal nodes of rabbits are a poor filter, an inferior barrier, to the escape of tubercle bacilli as compared with the sternal nodes.

CHANGES IN THE ADIPOSE TISSUE

1) Changes in the adipose tissue along the internal mammary vessels.-- Of particular interest was the constant occurence of various size and extent of tuberc-

ulous mass having no relation to the sternal lymphnodes, and their larger parts situated in the adipose tissues along the internal mammary vessels. These characteristic changes were most commonly seen in Group D, E and F, especially severe in Group F. Not only these tuberculous masses, but also the anterior portion of the chest wall which at necropsy appeared to be normal was removed as part of a block resection including the internal mammary chain, intercostal muscles, ribs, costal cartilages, and pleura, but the line of resection stopped at the sternal margin and was studied by serial section.

Microscopically, in all animals of Group D, E and F, somewhere along the internal mammary lymphatics the tuberculous foci were revealed. One animal which died 21 days after the first intraperitoneal inoculation revealed small epitheloid tubercles without caseation in various places limited to the surrounding adipose tissue along the internal mammary lymphatics.

One rabbit sacrificed from Group F, (one week after reinoculation) showed the same type of lesions, but these were much more extensive. In such cases the internal mammary lymphatics occupied the centre of a nodule of epitheloid cells with giant cells and these inflammatory processes spread peripherally, occasionally into the interstitial muscle tissue (Fig. 9). By the silver impregnation method, it becomes evident that there were constantly found at the site of reticulum fibers in which were seen the epitheloid cells and giant cells around the internal mammary lymphatics. In the former, there could be revealed the epitheloid cells mingling with the simultaneously injected carbon particles and tubercle bacilli in only the site of perivascular lymphinfiltration around the internal mammary lymphatics. This was accompanied by thrombosed lymphatics: the lumen often was blocked by a thrombus or by markedly proliferated endothelial walls, but not totally thrombosed. 1 month or more after reinoculation the section showed a significant lesion somewhere along these lymphatics. Grossly, the adipose tissue along the internal mammary chains demonstrated a few discrete nodular tubercles. These nodules ranged in size from about 100 μ to 2 mm. or more, the latter being visible to the naked eye. Microscopically, these tissues were largely replaced by small epitheloid tubercles, some of which had central caseation.

In some areas the tubercls were becoming confluent, with a large central focus of caseation. These tubercles permeated the interstitial tissue of the intercostal muscles and transversus thoracis. With appropriate stains acid-fast bacilli could be seen in these perivascular lesions. The walls of lymphatics were thick and cellular and the lumen encroached upon but not totally obstructed or thrombosed.

SUMMARY

We examined histologically the tissue intervening between submesothelial lymph capillaries of the internal mammary lymphatics and their regional superior sternal lymphnodes, and we could demonstrate the various size and character of the tuberculous lesions situated chiefly in the adipose tissue around these lymphatics. Without regard to the sternal lymphnodes, the presence of such tuberculous lesions at intermediate sites along the course of the internal mammary lymphatics

has never been observed. It seems that the specific physiological function of these lymphatics so-called leakage phenomenon may participate in the first development of such lesions. But, once extravascular lesion was established, these lesion might be proliferated and extended toward the surrounding muscles.

2) Changes in the adipose tissues beneath the parietal pleura.--In the anterior or posterior under portion of chest wall or the anterior portion of mediastinum, one can often see the goodly developed adipose tissue beneath the parietal pleura. In such a region an occasional tuberculous lesion could be found. As shown in figure 3, large tumors in the anterior portion of the mediastinum were revealed and their histological features afford typical isolated tuberculous granulomata with marked central caseation. In other cases a little tuberculous nodule in the adipose tissue beneath the posterior under portion of the parietal pleura. Histologically, various stages in the development of the tuberculous foci could be seen, from small, discrete collections of histiocytes engulfed with carbon particle, to the epitheloid cell tubercle that replaced normal structure by many more or less discrete aggregates of epitheloid cells with occasional LANGHANS' cells. Central caseation was occasionally found (Fig. 10). In some foci, many of round cell infiltrations and lymphinfiltration scattered at random, but these inflammtory processes tend to extended towards the surrounding tissue along the reticulum fibers. As a result of this finding, we are inclined to the conclusion that the tuberculous foci or tuberculoma are prone to develope in such adipose tissue beneath the parietal pleura.

CHANGES IN THE MACULA CRIBRIFORMIS (Fig. 11, 12, 13)

In 5 cases received with intrapleural inoculation, the varing size and extent of tumor were located in the lateral or posterior intercostal muscles (Fig. 2). These tumors were carefully removed enbloc with parietal pleura and studied by serial section. It showed microscopically the various stages of tuberculous foci from the large caseous, to the partly liquefaction of tumor with many polymorphnuclears.

Apart from these large tumor, encapsulated little caseous foci or tubercles in the thickened submesothelial collagen fibers of parietal pleura were seen, and often both tuberculous foci were connected by the lineal stretched round cell infiltrations. But these lineal round cell infiltrations spread along the reticulin fibers in the interstitial connective tissue of the intercostal muscles. Occasionally, it was seen obliterating tuberculous lymphangoitis in the adjacent submesothelial involvement. Then, to clarify the morphogenesis of these lesions, emphasis has been directed toward the morphologic changes in the early stage of these lesions.

Then some one week and others 2 weeks and three weeks respectively after intrapleural reinoculation, the animals were bled to death and a piece of parietal pleura riched in macula was carefully peeled and stained with BIELSCHOWSKY-MARESCH'S method and MAY-GIEMSA'S method. Results showed the following two types of behavior in relation to the macula cribriformis to tubercle bacilli invasion.

a) The structural element of the macula cribriformis invaded by tubercle bacilli consisted of severely altered collagen fibers. The collagen change was of the natu-

re of so-called fibrinoid swelling. The fibers became very thick, and somewhat wavy. These was replacement of fine reticulin fibrils by thick collagenous fibers. As a result of this fibrinoid swelling, the characteristic features revealed were the concentric luminal narrowing of macula cribriformis and no inflammatory cellular remnant (Fig. 14). While it has brought to light the fact that this concentric luminal narrowing of macula cribriformis is not necessarily of tuberculous origin but could be induced as an allergic response to horse serum. As a result of this observation, we are of the opinion that these marked features of macula cribriformis should be considered a state of defensive reaction resulting from an allergic response of parietal pleura to the protein fractions of the destroyed tubercle bacilli.

b) In contrast with above mentioned findings, in the instance of tubercle formation on the parietal pleura, from a comparatively early stage, it was characteristic feature that the macula cribriformis accompanied with proliferative reticulin fibrils was filled by aggregates of densely packed large mononuclears with engulfed carbon particles, lymphocytes, and a few cells with irregular pyknotic nuclei (Fig. 15). Slightly more advanced lesions to be confluent with each other, but without central caseation.

When these foci became older, they were gradually encapsulated by more thick proliferated collagenous fibers and gave the appearance of encapsulated caseous foci in the thick pleura (Fig. 16. 17).

In some instances, these enlarged foci invaded and broke out the elastic fibers beneath the parietal pleura, and then the inflammatory processes extended over intercostal muscles along the interstitial connective tissue (Fig. 18. 19. 20).

While, in the case of intraperitoneal inoculations, the peeled peritoneal diaphragm was replaced by the more marked geometrical regularly arranged tubercles revealing the evident remnant of the preexistent macula cribriformis (Fig. 21).

From above mentioned two findings, I considered this sort of tubercle to be the productive phase of inflammation due to tubercle bacilli that were caught by the macula cribriformis, the first barrier from the serous cavity to the lymphatic capillaries, taking place under conditions of allergic response.

SUMMARY:

The absorbed tubercle bacilli may come to a standstill in the first filterstations, i. e. macula cribriformis, forming circumscribed nodules or plaques. When these enlarge and become a focus of secondary dissemination, the danger of invasion of the chest wall or regional lymphnodes becomes imminent.

CHANGES IN THE PLEURAL ADHESIONS

The following experiment was attempted in order to investigate the bearing of chest wall of rabbits with experimentally induced pleurisy under high degree of allergy reactions: Intrapleural inoculation with horse serum—3 weeks later→Intrapleural inoculation with horse serum and tubercle bacilli—3 weeks later→intrapleural inoculation with tubercle bacilli 3 months later→autopsy. The result showed that at the inoculations site of pleural cavity the varying degree and spread of pleural adhesions such as caseous adhesion, scarred adhesion, cordlike adhesion between

parietal and visceral pleura but their predilections sites were not seen as in human beings and in generally there was a more marked tendency in the anterior portion.

Microscopically, these adhesions consisted of regular collagen fibers with relatively marked fibrosis that ran parallel to both pleura. It was a characteristic feature that there were seen a varying size of tubercles or capsulated caseated foci in these pleural adhesions. In various places adhesions were accompanied by little round cell infiltration or edema and fibrin exudate but these inflammatory processes could produce no rupture of subpleural elastic fibers. From the above mentioned experiments we could not catch the findings that these circumscribed tuberculous foci in the pleural adhesions were grown by the aggravation and subsequently broke through the parietal pleura then spread out towards the chest wall.

But it is of interest that these findings have suggested the results obtained by TAKEUCHI and by TSUZUKI and associates with clinical observations: i. e., that some pericostal tuberculosis which may be seen in form of peripleurisy has its origin in the growth of these localized foci in the pleural adhesions.

DISCUSSION

Concerning the pathogenesis of the pericostal tuberculosis the next two clinical facts have not been overlooked;

1) Concerning the predilection sites and frequency, it has been pointed out that the high proportion in the anterior and posterior under portion of the chest wall was obtained. This fact suggests that these regions receive anatomically somewhat a special limitation to the invasion of tubercle bacilli. The detailed anatomical studies of lymphatic system in this region may provides a clear explanation for this fact. Especially, the newer concepts of a so-called extra vascular fluid path that has been brought to light by KIHARA and his scholars make it more easy to explain this possibility.

2) During the past many years a close relation between the incidence of pericostal tuberculosis and idiopathic pleurisy or peritonitis has been firmly established by many workers. Viewed from a statistical angle in our clinic in the subsequent history of patients with them, at least 86% of these cases seems particularly prone to arise within six monthes following demonstrable idiopathic pleurisy or peritonitis. Viewed from a clinical course, idiopathic pleurisy afford the following generally accepted features; the MANTHOUX test is usually highly positive directly prior to the occurrence of idiopathic pleurisy but suddenly tends to decline with the occurrence of them and occasionally becomes negative in the severe case. But this decline tendency becomes positive in accordance with the decay of them but more marked degree than in the foregoing occurence, then becomes same degree as it was. Taking advantage of this opportunity in the cases revealed aggravation of the foci in the lung the reaction does not tend to intensify.

With improved methods of culturing pleural fluid the number of positive cases is revealed in about 100 per cent at the onset of idiopathic pleurisy. And in occurrence with the decay of disease the tubercle bacilli in the effusion tend to

indicate the negative result of cultures, but occasionally a positive result is obtained followed this negative phase.

From the above mentioned fact it is evident that; idiopathic pleurisy is the result of the allergy reaction due to tubercle antigen and at the same time as a result of hyperallergic reaction the tubercle bacilli are temporally destroyed in the larger proportion of cases. Occasionally the tubercle bacilli as excessive antigen gradually multiply in availing with this chance because it becomes an anergic state. As it is able to consume the antibody, the anergic state develops and consequently the tubercle bacilli, as an excessive antigen, gradually multiply resulting in the aggravation of lesion.

While, in the former instances, the destroyed bacilli are destined to be absorbed with the effusion or tissue debris.

Nowadays, concerning the absorption from the pleural cavity it is generally accepted that fluids are absorbed chiefly by the blood-vessels and that particulate matter is absorbed chiefly through lymph channels.

Then these destroyed bacilli and tissue debris are removed through the lymph channels, and consequently lead to the changes in the macula or tributary lymph nodes. On the first, the protein fraction of the destracted tubercle bacilli may induce the nature of so-called fibrinoid swelling resulting the concentric luminal narrowing of macula cribriformis. These events are induced by the another sort of protein i. e. ovum-albumin, horse serum. Since the development of the histochemistry and the electromicroscope, considerable work has been done on the ultrastructure of collagen fibrils from various normal and allergic state, and recently the fibrinoid swelling under tuberculin allergy state has been investigated. Though many unsolved problems remain in this respect, these studies give some suggestion on the fibrinoid sweeling of the macula cribriformis. While, the representative changes in the tributary lymphnodes seem to be relatively slight revealing the sinus cattarrh of large cellular hyperplasia. In contrast with this, in the occasion of latter, multiplied tubercle bacilli often become lodged at first in the lumen of macula cribriformis and small luminal tubercles are formed. In some cases these miliary luminal tubercles grow to large caseous nodes, thus resulting the spread of these processes to the surrounding tissues accompanying with obliterating tuberculous lymphangoitis. If allowed these multiplied tubercle bacilli to reach to the tributary lymphnodes, we can reveal the relative severe changes such as caseous lymphadenitis, in which all normal architecture has been destroyed, or diffuse caseous lymphsinuitis, or solitary tubercles etc.

To account for the clinical evidence that the anterior portion of chest wall show a high incidence of them and apart from them, occasionally it is accompanied with the occurence of two or three isolated tumors on each site having regardless of and no histological relation to the ordinarly situated sternal nodes, it is difficult only with the generally accepted view that the majority of them in the anterior portion have their origin in sternal lymphnodes. In fact, in a series of 60 postmortem human examinations STIBBE found the average total number of sternal

lymph nodes per subject to be 8.5 and their typical distribution was four on one side and five the other. More recently this distribution was confirmed by many workers on the studies of carcinomatous metastasis.

While in man as well as animals, there are arranged with many primitive lymphatic apparatuses such as lymphnodules or lymphinfiltration stringing out along the internal mammary lymphatics as pointed out by KIHARA and his scholars. But it seems that such primitive lymphapparatuses as well as the exsistence of reticulin fibers offer the site of leakage phenomenon and at the same time can be the site of inflammation.

As a matter of fact, a satisfactory reproducible tuberculous lesion in the adipose tissue along the internal mammary lymphatics described in the present report could be caused and indicated no relation to the ordinarily situated sternal lymph nodes.

In order to establish a tubercle in such a region it is thought to be of vascular origin unless by direct injection.

It seems to me that in order to establish a tubercle, the tubercle bacilli must pass through the endothelial barrier and be provided fortunate circumstances to survive for prolonged periods of time within such tissues. Concerned with the former the specific function of the so-called leakage phenomenon provides a clear explanation for this fact. As to the latter, I myself have seen no histochemical evidence of an antituberculous property of these tissues. The adipose tissue appeared rather to be a highly fertile soil for the growth of most tubercles. For this reason, it is thought that on the first these tissues are rich in vascular system and formed the extravascular fluid path, and secondly rich in various sorts of fat having close relation to the metabolism of tubercle bacilli.

Their responsibility rests upon the specific physiological function of the internal mammary lymphatics as if it is clear from the fact that it can be caused by the remarkable lesion centered on the internal mammary lymphatics tending to spread only along the path of reticulin fibers toward the surroundings, when employed with the intraperitoneal injection, but only the poor tubercle employed the blood born injection with the goodly large quantity of tubercle bacilli as mentioned later.

Finally, it appears from our material that tubercle bacilli are capable of proliferating in the adipose tissues surrounding the internal mammary lymphatics at the site of arrest, as shown by the development of the epitheloid tubercle, or caseous tubercle.

Since the tuberculous foci in the adipose tissues beneath the parietal pleura are readily demonstrable in such serous inflammatory course, it became of interest to determine whether they are due to blood-born or lymphogenous infection. However, we hold that such lesion is not the direct result of septicaemia with tubercle bacilli, but that it's development depends upon the absorption from the pleural cavity to the lymphatics in such adipose tissue.

We reach this conclusion by the following evidence:

1) the appearance of carbon particles in such adipose tissues can be revealed

only by the blood-born injection of a sufficiently large quantity of India ink (pro Kg. of rabbits) to leakage in such adipose tissue from the venule.

2) under physiological circumstances the India ink introduced pleural cavity are absorbed into only the lymphatic vessels in such subpleural adipose tissue, but not venule.

3) experimentally, the occurence of instances of extreme, generalized miliary tuberculosis with a few poor lesion in such adipose tissues.

SUMMARY

A satisfactory reproducible pericostal tuberculosis could be caused and histologically it could be revealed the following types of development.

1) Pericostal tuberculosis, in part at least, arise from liquefying lymphnodes which were infected from pleura or abdominal cavity. Such types of incidence were the most prominent in the group of sternal lymphnodes.

2) The extravascular leakage phenomenon that is the specific physiological function of the internal mammary lymphatics may participate, at least in partly, in the development of pericostal tuberculosis in the anterior portion of the chest wall.

3) Histological studies on serial section and peeled extension preparate enabled us to determine that some of growths in the intercostal muscles originated from the primary foci caused as a sequele of detention in the macula cribriformis.

4) What appears to be pericostal abscess may be, in part, due to foci in pleural adhesions, but more often arises from the foci in the macula cribriformis.

As a result of this study, we are inclined to the conclusion that pericostal tuberculosis is widerspread tuberculosis of the lymphatic system of the chest wall, induced by the absorption of tubercle bacilli from serous cavity as a sequel of idiopathic pleurisy or peritonitis.

Fig. 1. Gross appearance of tumor in the anterior portion of the chest wall.

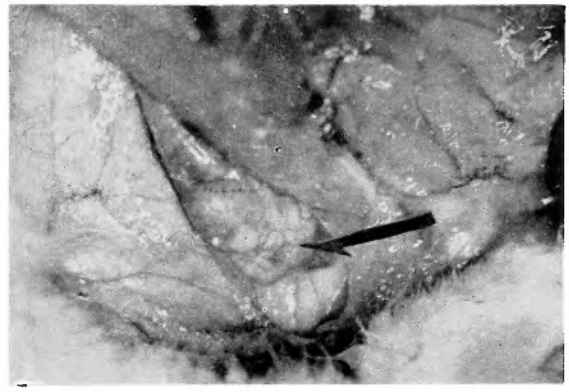

Fig. 2. Gross appearance of tumor in the dorso-lateral portion of the chest wall.

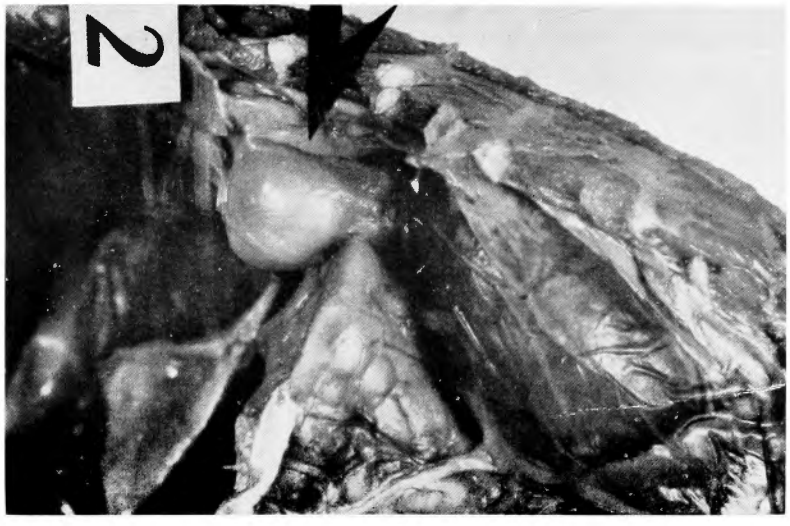

Fig. 3. Gross appearance of tuberculous mass formation between both mediastinum.

Fig. 4. Section from the superior sternal lymphnodes.

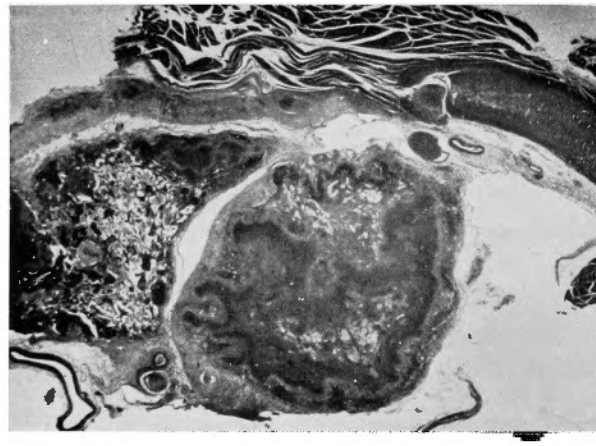

Fig. 5. Section from the superior sternal lymphnodes.

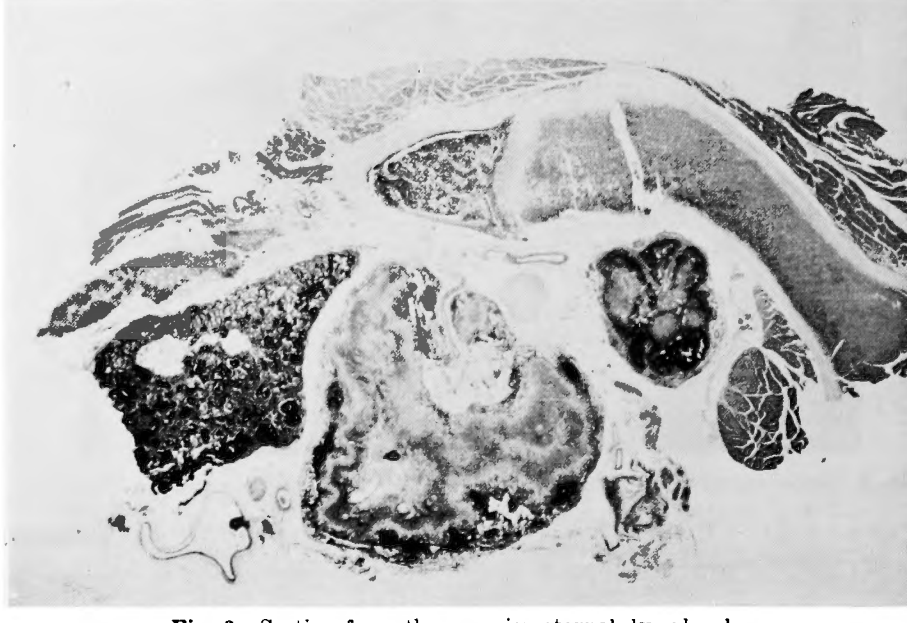

Fig. 6. Section from the superior sternal lymphnodes.

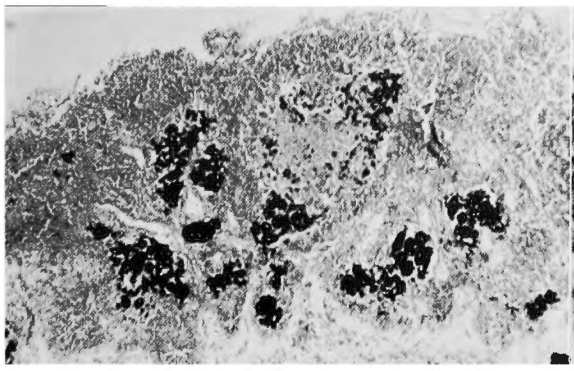

Fig. 7. The isolated productive foci chiefly laid in the medullary sinuses partly encroaching upon the medullary cords.

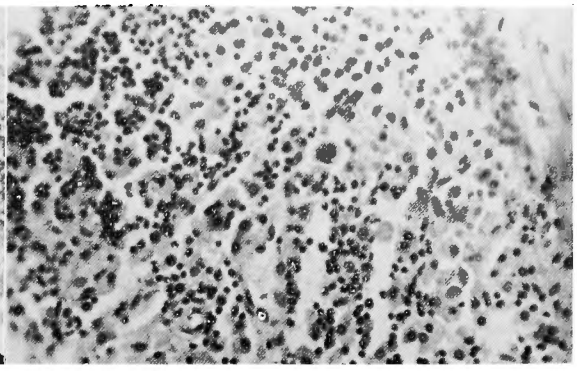

Fig. 8. Diffuse epitheloid cell proliferating lymphosinuitis. (so-called large cellular hyperplasia)

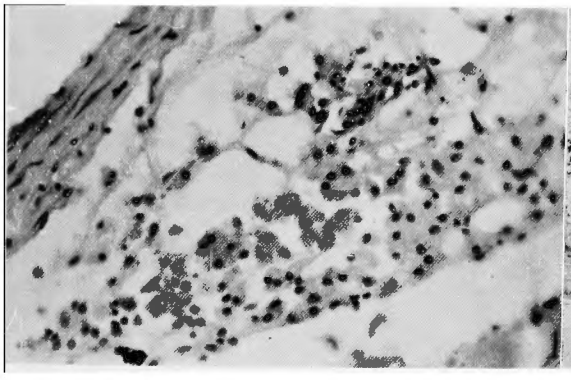

Fig. 9. The epitheloid cell tubercles limited to the surrounding adipose tissue along the internal mammary lymphatics.

Fig. 10. The tuberculous nodule in the adipose tissue beneath the parietal pleura.

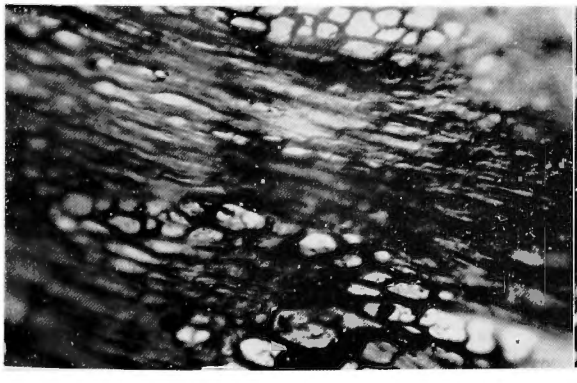

Fig. 11. Preparation from the peeled parietal pleura of the rabbit showing the normal Macula cribriformis. (anterior under portion) ×400

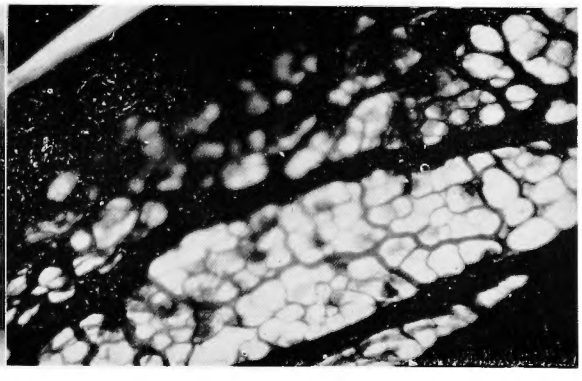

Fig. 12. Preparation from the peeled parietal pleura of rabbit showing the normal Macula cribriformis. (posterior under portion) ×400

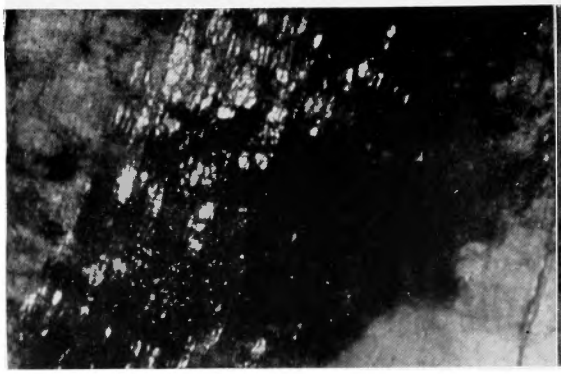

Fig. 13. The normal Macula cribriformis of the rabbit. ×100

Fig. 14. The concentric luminal narrowing of Macula cribriformis due to tuberculous origin.

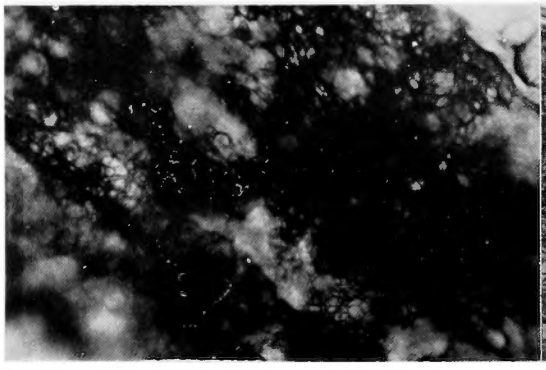

Fig. 15. The Macula cribriforrmis accompanied with proliferative reticulin fibrils.

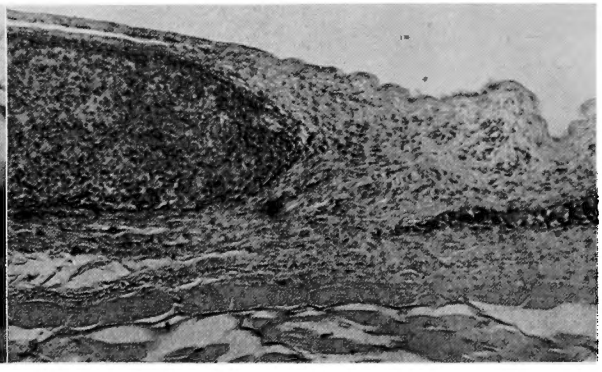

Fig. 16. The appearance of encapsulated caseous foci in the thick pleura.

Fig. 17. The gross appearance of encapsulated caseous foci in the posterior under portion of the parietal pleura.

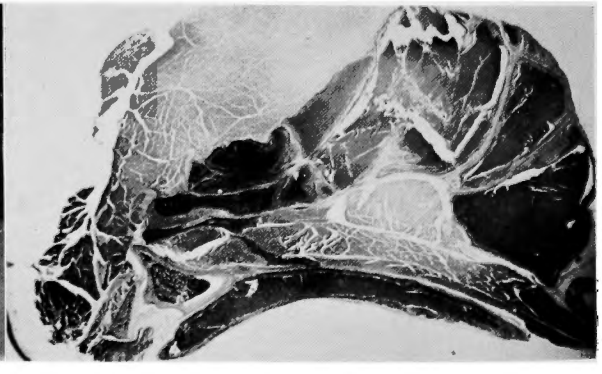

Fig. 18. The encapsulated caseous foci enlarged and invaded the elastic fibers beneath the parietal pleura, and extended over intercostal muscles.

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Fig. 19. The inflammatory processes extended over intercostal muscles along the interstitial connective tissue. Hematoxylin and eosin stain. ×100

Fig. 20. same as Fig. 19. BIELSCHOWSKY-MARESCH's silver impregnation's methode. ×100

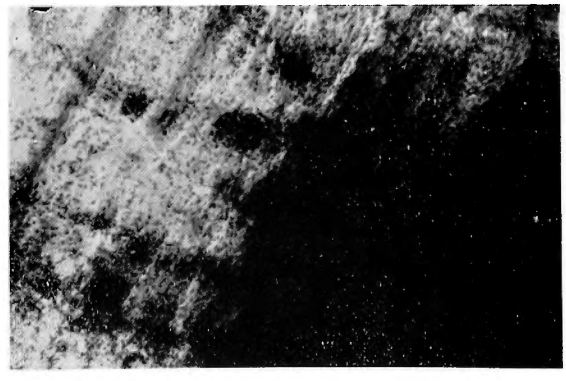

Fig. 21. The regularly arranged tubercles revealing the remnant of the preexistent Macula cribriformis of the peritoneal diaphragm. Hematoxylin and eosin stain. ×100

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和 文 抄 録

胸囲結核症の発生に関する実験的研究

京都大学医学部外科学教室第2講座（青柳安誠教授　指導）

大学院学生　山　本　政　勝

胸囲結核症の成因として，我々は両胸膜間の癒着部に新生されたリンパ系流を経て，肺内結核菌が胸壁リンパ系を侵すか，いま一つは胸腔内滲出液中の結核菌が直接胸壁胸膜から吸収されて胸壁リンパ系を侵すものであろうと提唱して来た．

私は特に後者の場合を実験的に吟味した．即ち人型結核菌感作実験によつて，種々の程度の胸腹膜炎を家兎に惹起せしめその後胸壁リンパ系に起る変化を組織学的に追究した．

まず本実験に先立ち，健常家兎，同モルモットの胸腔内竝びに腹腔内からの淋巴系吸収経路を墨汁注入法及び吸収法によつて確めた結果，木原教授一門の研究竝びに坂本，Jossifow，志田氏等の所見と一致した．

特に，木原教授の所謂前リンパ管通液路を構成する篩状斑竝びに，旁リンパ管通液路の見られる内胸リンパ管が，本症の発生に特に密接な関係を有する事を知り，之等の形態，機能分布等について詳細に検索した．

次で前述の実験家兎を逐次的に剖検すると，種々なる程度の結核性腫瘤を胸壁に認めたが，前胸部に発生したものと，後側胸部に発生したものとは，その様相を多少異にするように思われた．そこで，之を組織学的に検索すると，前胸部に於ける腫瘤は明かに，胸骨リンパ節が全体として乾酪化したものや，なお一部に正常リンパ組織の残存するものから，洞カタルに至るまで種々な程度の結核性リンパ節炎の像を呈していた．更にかゝる変化は，篩状斑発現様式をとつているものと，なお腹腔内感作動物に於ては，内乳リンパ管に沿うて処々脂肪組織内に結核結節を認め得た点から，木原教授のもとで，手島博士によつて発見された内乳リンパ管の漏出現像というそのもつ特殊性からも発現し得ることが判明した．

後側胸部に於けるものは，篩状斑出現が主で，吸収された菌が篩状斑にひつかゝつて，結節を形成するが，その中には爾後増大して胸膜周囲膿瘍を形成するまでに発展する型と，篩状斑に於けるかゝる結節から二次的に筋間結合織内をリンパ行性に進み肋間筋内に大きな膿瘍を形成する型のあることを明かにし得た．この際家兎では側肋間リンパ節が認められないので，リンパ節の変化は確認し得なかつた．又この際一般的に云つて後肋間リンパ節の変化は軽微であつた．

以上の実験結果から，胸囲結核症は結論的に胸壁リンパ系の結核であると云い得るし，胸腔内，時には腹腔内の結核菌が特定部位から胸壁リンパ系に吸収されても発生し得ると考えて間違がないであろう．